Synthesis and fungicidal activity against *Pyricularia oryzae* of some heteroaryl thiophene carbamates†

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Abstract: A series of novel 3-(5-cyanopentylcarbamoyloxy)-5-(heteroaryl)-thiophene-2-carboxylic acid ester and amide derivatives has been synthesised and tested for fungicidal activity against the rice blast pathogen *Pyricularia oryzae*. The highest levels of fungicidal activity were observed when the heteroaryl group comprised either a 2-pyridyl or 2-pyrazinyl group. Ester derivatives proved to be more active than amides, the highest activity being associated with esters derived from C_6 and C_7 alcohols.

Keywords: pyridylthiophene; fungicide; rice blast; *Pyricularia oryzae*

1 INTRODUCTION

As part of our research programme to identify new agricultural fungicides, we became interested in a series of thiophene dicarboxylic acid derivatives of type **1** (Fig 1) which were reported to control the rice blast fungus, *Pyricularia oryzae* Cav, the major fungal disease of rice in Japan and South East Asia.[1,2] Using structure **1** as a lead, a range of novel compounds were synthesised and tested. Initially this consisted of fused biheterocyclic systems[3] but we subsequently extended our investigations to include a range of linked biheterocyclic systems with the general structure **2** (Fig 2), which are the subject of this paper.

2 MATERIALS AND METHODS

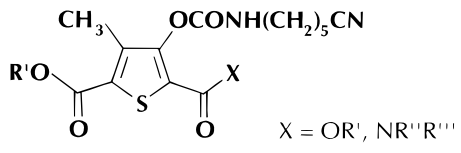
2.1 Synthetic procedures

[^{1}H]NMR spectra were determined with a Bruker AC300 spectrometer using deuterochloroform as the solvent. Chemical shifts are given in parts per million relative to tetramethylsilane as standard. Elemental analyses were performed on a Carlo-Erba 1106 instrument. All melting points are uncorrected.

The general synthetic route to the heteroaryl thiophene carbamates (**2**) is depicted in Fig 3 and entails a novel reaction for the formation of the thiophene nucleus, involving nucleophilic displacement of diethyl phosphate from the unsaturated ester (**3**) by a thiolate anion to give the thioester intermediate (**4**) followed sequentially by a Dieckmann cyclisation[4] to the hydroxythiophene (**5**) and carbamoylation. Unsaturated esters (**3**) are obtained by reacting the appropriate β-ketoester, derived by standard Claisen condensation chemistry,[5] with diethyl phosphorochloridate under basic conditions. The carbamates (**2**) prepared in this way are presented in Table 1. A typical synthetic procedure, for cyclohexyl 3-(5-cyanopentylcarbamoyloxy)-5-(2-pyridyl)-thiophene-2-carboxylate (**2a**), is given as an example.

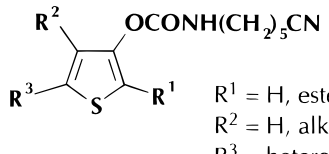
2.1.1 *Cyclohexyl 3-(5-cyanopentylcarbamoyloxy)-5-(2-pyridyl)-thiophene-2-carboxylate* (***2a***)

Sodium hydride (5.41 g; 600 g litre^{-1} in oil) was added to a stirred solution of ethyl 3-(2-pyridyl)-3-



(**1**)

Figure 1. Rice blasticidal thiophene derivatives reported in 1987 (References 1, 2).



(**2**)

Figure 2. General structure for novel linked biheterocyclic compounds.

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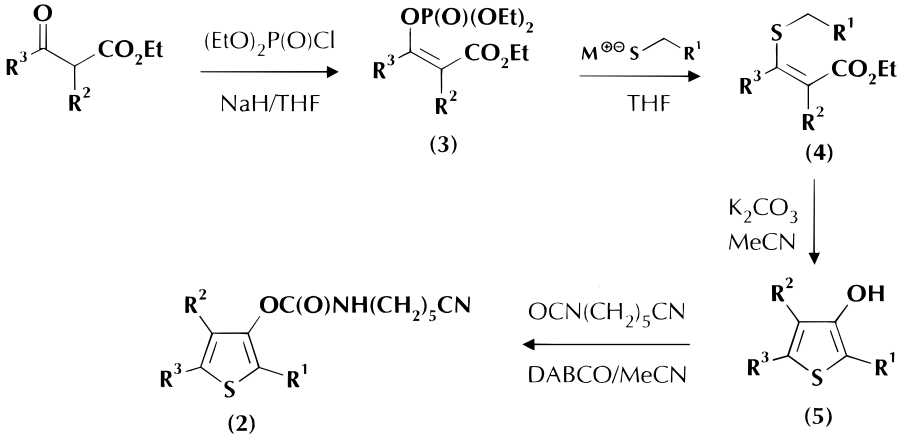


Figure 3. General synthetic route to heteroaryl thiophene carbamates (**2**).

oxopropanoate (25.80 g) in tetrahydrofuran (200 ml) cooled to 0°C. The mixture was stirred for 30 min, then diethyl phosphorochloridate (23.17 g) in tetrahydrofuran (130 ml) was added over 15 min. After stirring for 4 h at room temperature, the mixture was poured into brine and extracted with ethyl acetate. The ethyl acetate extracts were dried over magnesium sulfate and the solvent removed *in vacuo* to give a quantitative yield of crude ethyl 3-(diethoxyphosphinyloxy)-3-(2-pyridyl)prop-2-enoate (**3**; $R^2 = H$; R^3 = 2-pyridyl) as an oil (47.98 g).

Sodium hydride (2.44 g; 600 g litre^{-1} in oil) was added to a stirred solution of cyclohexyl mercaptoacetate (10.61 g) in tetrahydrofuran (160 ml) at 0°C and the mixture stirred at 0°C for 40 min. A solution of ethyl 3-(diethoxyphosphinyloxy)-3-(2-pyridyl) prop-2-enoate (**3**; R^2, R^3 as above; 20.00 g) in tetrahydrofuran (50 ml) was then added over 35 min at 0°C. After stirring for 4 h at room temperature, the mixture was poured into brine and extracted with ethyl acetate. The ethyl acetate extracts were dried over magnesium sulfate, the solvent removed *in vacuo* and the oily residue chromatographed on silica gel. Elution with mixtures of ethyl acetate + hexane gave ethyl 3-(cyclohexyloxycarbonylmethylthio)-3-(2-pyridyl)prop-2-enoate as an oil (**4**; $R^1 = CO_2c\text{-}C_6H_{11}$; $R^2 = H$; R^3 = 2-pyridyl; 15.02 g; 67.4%).

A stirred mixture of ethyl 3-(cyclohexyloxycarbonylmethylthio)-3-(2-pyridyl)prop-2-enoate (**4**; R^1, R^2, R^3 as above; 13.49 g), potassium carbonate (5.11 g) and acetonitrile (220 ml) was heated under reflux for 7 h. The cooled mixture was filtered and the solid was washed with acetonitrile and then dissolved in water (300 ml) and acidified with hydro-

Table 1. Heteroaryl thiophene carbamates (**2**) synthesised for bioassay[a]

Compound No	R^1	R^2	R^3	*Yield* (%)	*mp* (°C)
2a	$CO_2c\text{-}C_6H_{11}$	H	2-pyridyl	48.6	90–91.5
2b	$CO_2CH_2c\text{-}C_6H_{11}$	H	2-pyridyl	15.8	76.4–77.5
2c	$CO_2CH_2C_6H_5$	H	2-pyridyl	33	83.5–86
2d	$CO_2C_7H_{15}$	H	2-pyridyl	37	74.6–75.6
2e	$CO_2CH(CH_3)C_3H_7$	H	2-pyridyl	42	oil
2f	$CO_2CH(CH_3)_2$	H	2-pyridyl	49	86–88
2g	$CON(CH_2)_5$	H	2-pyridyl	89	120–123
2h	$CON(CH_3)c\text{-}C_6H_{11}$	H	2-pyridyl	63	134–136
2i	$CONHC_6H_5$	H	2-pyridyl	48.3	139–141
2j	$CO_2c\text{-}C_6H_{11}$	CH_3	2-pyridyl	77.8	90–91.5
2k	$CO_2c\text{-}C_6H_{11}$	OCH_3	2-pyridyl	64.2	85–86
2l	$CO_2c\text{-}C_6H_{11}$	$N(CH_3)_2$	2-pyridyl	25	oil
2m	$CO_2c\text{-}C_6H_{11}$	SCH_3	2-pyridyl	68	136–137
2n	$CO_2c\text{-}C_6H_{11}$	H	3-pyridyl	5.7	oil
2o	$CO_2c\text{-}C_6H_{11}$	H	4-pyridyl	26	76–80
2p	$CO_2c\text{-}C_6H_{11}$	H	2-pyrazinyl	27	146.5–149.5
2q	$CO_2c\text{-}C_6H_{11}$	H	C_6H_5	16	83–84
2r	$CO_2c\text{-}C_6H_{11}$	H	2-furyl	22	73–76
2s	$CO_2c\text{-}C_6H_{11}$	H	5-(CH_3)-2-thienyl	49	85–87

[a] For general formula see Fig 3.

chloric acid (2 M). The mixture was extracted with dichloromethane, the extracts dried over magnesium sulfate and the solvent removed *in vacuo* to give cyclohexyl 3-hydroxy-5-(2-pyridyl)thiophene-2-carboxylate (**5**; $R^1 = CO_2c\text{-}C_6H_{11}$; $R^2 = H$; $R^3 = 2$-pyridyl) as a cream solid (5.75 g; 49.1%) which was then converted, according to the carbamoylation method reported for compounds of type **1**,[1,2] to cyclohexyl 3-(5-cyanopentylcarbamoyloxy)-5-(2-pyridyl)thiophene-2-carboxylate (**2**; $R^1 = CO_2c\text{-}C_6H_{11}$; $R^2 = H$; $R^3 = 2$-pyridyl; **2a**). Yield 48.6%; mp 90–91.5°C. [^{1}H]NMR δ: 1.33–1.94 (16H, m), 2.41 (2H, t), 3.30 (2H, q), 4.97 (1H, m), 5.34 (1H, t), 7.24 (1H, m), 7.50 (1H, s), 7.67 (1H, m), 7.74 (1H, m), 8.59 (1H, d). Analysis found: C, 62.87; H, 6.18%. Calculated for $C_{23}H_{27}N_3O_4S$: C, 62.56; H, 6.16%.

2.2 Biological assay

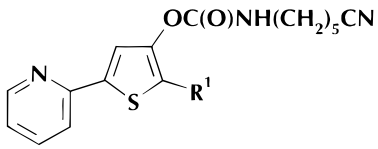
All compounds were tested for control of the rice blast pathogen, *P oryzae*, on rice seedlings (*Oryza sativa* L) var. Nihonbare at the first-leaf stage. The compounds were formulated in water + acetone (3 + 2 by volume) containing 2.5 g litre^{-1} Tween 20 to give 125 and 25 mg litre^{-1} solutions which were applied to the rice seedlings as a foliar spray using a hand-held spray gun. After 24 h on drying racks under lights, the seedlings were inoculated with spores of *P oryzae* (4×10^4 spores ml^{-1}) in distilled water containing Tween 20 surfactant (125 mg litre^{-1}), using a hand-held spray gun. The *P oryzae* spores were derived from infected live plant material upon which spore production had been induced. The plant material was agitated in distilled water containing Tween 20 (125 mg litre^{-1}) to dislodge the spores and the mixture filtered through muslin to remove the plant matter. The conidial concentration of the filtrate was measured using a haemocytometer and was adjusted to 4×10^4 spores ml^{-1} by dilution. The seedlings were kept in the dark for 24 h at 26°C and were then incubated for 6–9 days at 21°C under a regime of 16 h light and 8 h darkness per day. The seedlings were assessed 7 to 10 days after inoculation for the extent of lesion formation on the leaves, using a scale 0 to 4 where 0 = 0–24%; 1 = 25–49%; 2 = 50–74%; 3 = 75–99% and 4 = 100% control, compared with the control which consisted of plants which had received aqueous Tween 20 and subsequently been inoculated with the test fungus.

3 RESULTS AND DISCUSSION

Optimal rice blasticide activity of compounds **2** was associated with an ester functionality at R^1 and, more specifically, esters derived from aliphatic or alicyclic C_6 and C_7 alcohols. However, the ester derived from benzyl alcohol was inactive. Amide derivatives displayed inferior activity compared to ester derivatives (Table 2).

For the R^2 group, hydrogen proved to be the preferred substituent. Other small substituents resulted in some decrease in activity, with the exception of the methylthio group, which completely abolished the rice blasticide activity (Table 3).

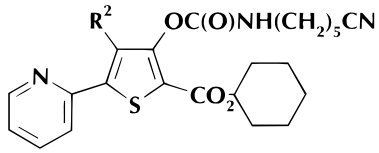
The preferred substituent for R^3 was found to be 2-pyridyl (or 2-pyrazinyl). Loss of biological activity was observed if the point of attachment of the pyridine ring to the thiophene ring, was changed to the 3- or 4-pyridyl position. Removal of the nitrogen atom, by replacement of the pyridyl ring with a



Compound No.[a]	R^1	*Disease incidence score*[b] *when applied at dose (mg litre^{-1})*	
		125	*25*
2a	$CO_2c\text{-}C_6H_{11}$	4	4
2b	$CO_2CH_2c\text{-}C_6H_{11}$	3	3
2c	$CO_2CH_2C_6H_5$	0	i
2d	$CO_2C_7H_{15}$	4	4
2e	$CO_2CH(CH_3)C_3H_7$	4	2
2f	$CO_2CH(CH_3)_2$	1	i
2g	$CON(CH_2)_5$	3	1
2h	$CON(CH_3)c\text{-}C_6H_{11}$	3	1
2i	$CONHC_6H_5$	0	i

Table 2. Rice blasticide scores for compounds **2** ($R^2 = H$; $R^3 = 2$-pyridyl).

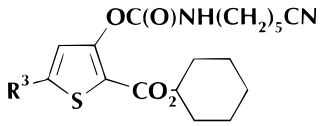
[a] See Table 1.
[b] On a scale 0–4 where 0 = 0–24; 1 = 25–49; 2 = 50–74; 3 = 75–99% control and 4 = complete control; i = inactive at this dose.



Compound No.[a]	R^2	*Disease incidence score*[b] *when applied at dose (mg litre^{-1})*	
		125	*25*
2a	H	4	4
2j	CH_3	3	i
2k	CH_3O	3	2
2l	$(CH_3)_2N$	3	2
2m	CH_3S	0	i

Table 3. Rice blasticide scores for compounds **2** ($R^1 = CO_2c\text{-}C_6H_{11}$; R^3 = 2-pyridyl)

[a] See Table 1.
[b] On a scale 0–4 where 0 = 0–24; 1 = 25–49; 2 = 50–74; 3 = 75–99% control and 4 = complete control; i = inactive at this dose.



Compound No[a]	R^3	*Disease Incidence Score*[b] *when applied at dose (mg litre^{-1})*	
		125	*25*
2a	2-pyridyl	4	4
2n	3-pyridyl	4	3
2o	4-pyridyl	0	i
2p	2-pyrazinyl	4	4
2q	C_6H_5	3	1
2r	2-furyl	1	i
2s	5-(CH_3)-2-thienyl	2	i

Table 4. Rice blasticide scores for compounds **2** ($R^1 = CO_2c\text{-}C_6H_{11}$; R^2 = H)

[a] See Table 1.
[b] On a scale 0–4 where 0 = 0–24; 1 = 25–49; 2 = 50–74; 3 = 75–99% control and 4 = complete control; i = inactive at this dose.

Compound No[a]	*Disease incidence score*[b] *when applied at dose (mg litre^{-1})*	
	125	*25*
2a	4	4
1 (X = $OCH(CH_3)_2$; $R^1 = CH(CH_3)_2$)	4	2
1 (X = $N(CH_2)_5$; $R^1 = CH(CH_3)_2$)	3	1

Table 5. A comparison of rice blasticide scores for compounds **2a** and **1**.

[a] See Table 1.
[b] On a scale 0–4 where 0 = 0–24; 1 = 25–49; 2 = 50–74; 3 = 75–99% control and 4 = complete control.

phenyl group, resulted in loss of activity, as did replacement with either 2-furyl or 2-thienyl groups (Table 4).

In a comparative test, dicarboxylic acid derivatives of type **1**, the lead compound, were subjected to the same biological screen. Their activity was seen to be inferior to that of the best of the new compounds, particularly at the 25 mg litre^{-1} dose rate, where compound **2a**, for example, unlike compound **1**, continued to give complete control of the rice blast pathogen (Table 5). However, efforts to transfer compound **2a** to the field were disappointing for reasons which remain obscure.

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